November 29, 2006

Dr. William S. Stokes
NICEATM Director
NIEHS
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RE: Public comment to the NICEATM Pre-Screen Evaluation of the *In Vitro* Endocrine Disruptor Assay (Robotic MCF-7 Cell Proliferation Assay of Estrogenic Activity), October 16, 2006, Federal Register Notice (Vol. 71, No. 199 pp. 60748-9 (71FR0748)).

Dear Dr. Stokes:

The American Chemistry Council (ACC or the "Council") has played an active role in the development and implementation of the EPA's endocrine disruptor screening and testing program (EDSP) for several years.[1] The Council supports the EPA's efforts to validate endocrine disruptor screening and testing methods and ACC looks forward to the timely development and implementation of a scientifically sound EDSP. The Council represents more than 90 percent of the productive capacity for basic industrial chemicals within the United States and its members are the leading companies engaged in the business of chemistry.

EPA's EDSP may significantly affect the Council and its members. For that reason, the Council and its members have attempted to assist the EPA in developing and implementing its EDSP. In that regard, ACC and its members actively participated in EPA's Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) and

[1] The American Chemistry Council represents the leading companies engaged in the business of chemistry. ACC members apply the science of chemistry to make innovative products and services that make people's lives better, healthier and safer. ACC is committed to improved environmental, health and safety performance through Responsible Care, common sense advocacy designed to address major public policy issues, and health and environmental research and product testing. The business of chemistry is a $460 billion enterprise and a key element of the nation's economy. It is the nation's largest exporter, accounting for ten cents out of every dollar in U.S. exports. Chemistry companies invest more in research and development than any other business sector. Safety and security have always been primary concerns of ACC members, and they have intensified their efforts, working closely with government agencies to improve security and to defend against any threat to the nation's critical infrastructure.

are actively participating in EPA's current methods validation technical advisory committee. Although the MCF-7 assay was discussed by EDSATC, it was not included in the committee's final list of recommended assays and currently it is not slated for inclusion in EPA's EDSP. However, since it has been brought forward to the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), we appreciate the opportunity to submit comments on NICEATM's Pre-Screen Evaluation. Validation is an important issue for assays under consideration as endocrine screens, and one to which the American Chemistry Council and its member companies have given considerable attention. We appreciate this venue for participation, with the intent to provide thoughtful and constructive perspectives helpful to NICEATM in undertaking the validation process of the Robotic MCF-7 Cell Proliferation Assay of Estrogenic Activity submitted by Certi Chem, Inc. (CCi).

We recognize that the NICEATM Pre-Screen Evaluation of the subject assay is but an initial step in the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM's) process of nominating and validating new test methods, and that there may be many additional opportunities for review and comment at subsequent steps in the process. Nonetheless, our interest is in providing detailed comments responsive to the FR notice of October 16. To do this, we would need access to the information provided in CCi's Background Review Document (BRD), yet such information was not made available. Without that information, it is not possible to evaluate the submission itself and to comment meaningfully on NICEATM's evaluation. We understand your office plans to provide access to BRDs submitted for assays in the future, and we encourage such an open access policy to enable more complete review and public comment.

For the subject FR Notice, therefore, we are able to provide only general comments due to the paucity of detailed technical information available. Some specific points follow.

1. NICEATM's summary of the BRD information on accuracy, reliability, and data quality of the proposed test method (Sections 2.2, 2.3, & 2.4) provide little detailed information and are insufficient to comment on, however, the deficiency of information on statistical analysis to investigate intra- and inter-laboratory reproducibility certainly raises concerns (Section 2.4.1).

2. With respect to the evaluation of concordance in NICEATM's pre-screen evaluation of this assay (Section 2.4.3), the presentation of some material is potentially misleading. For example, Table 2 leaves the impression that ICCVAM has reviewed and approved the data submitted by CCi, rather than simply that the data have been provided by CCi and compared to ICCVAM's data on reference substances. This distinction is critical, particularly given the inability to review the actual data provided in CCi's BRD. Although we realize the distinction has been mentioned in the document, we suggest it be repeated and made more prominent. It is especially important to be clear that the NICEATM pre-screen evaluation of CCi's Robotic MCF-7 Cell Proliferation Assay of Estrogenic Activity does not address the quality of the data generated by CCi.

3. With respect to NICEATM's summary statement that "The CCi BRD also adequately addresses the performance of the CCi test method," we feel that a summary of the limitations and deficiencies noted in the previous sections should also be provided here.

4. Because we are unable to review CCi's BRD, we base some general comments on the published literature on the MCF-7 cell proliferation/E-Screen assay and the recommendations of the Endocrine Disruptor Screening and Testing Committee (EDSTAC).
    a. The E-Screen assay was not selected by EDSTAC because of specific concern that the proliferative response is indirect, i.e. the presence of a functional estrogen receptor is necessary, but not sufficient to evoke estrogen-mediated cell proliferation. EDSTAC noted that it may be difficult to standardize this assay for large-scale testing. Most troublesome is EDSTAC's concern that the E-Screen assay may identify general cell mitogens, growth factors, and some steroids as false positives and growth inhibitors or cytotoxicants as false negatives. (EDSTAC Report, 1998). It will be important for the validation effort to resolve these concerns.
    b. The published literature illustrates that concerns about the E-Screen assay include:
        i. False positives have been demonstrated for the solvent ethanol, growth factors, and some steroids. One report could not re-generate positive results upon retesting an unopened batch of ethanol and concluded that the original positive proliferation effects must have resulted from contamination of the ethanol with an estrogenic substance (Andersen et al. 1999). Another investigation reported positive results with ethanol, progesterone, and epidermal growth factor indicating that cell proliferation may be inducible by a mechanism other that ER binding in the MCF-7 cell line (Jones et al. 1998).
        ii. The group of investigators nominating the E-Screen assay have continuously reported that no false positive results are generated with this assay (Soto et al. 1995, Soto et al. 2006, Sonnenschein et al. 1995), despite reports to the contrary noted above and the conclusion of the subject document (see page 13, Table 2).
        iii. Chemicals that are hydrophobic or have high vapor pressure are difficult to evaluate in multi-well culture plates; volatile compounds may generate false positives in nearby control wells (DeCastro et al. 2006).
        iv. There is disagreement in results generated by chemicals with modes of action different than direct binding to the ER (Jones et al. 1998, Andersen et al. 1999). This assay cannot be fully validated until unequivocal evidence exists that a cell line will not proliferate except when test substances bind to the ER (Jones et al. 1998).

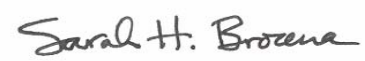
Without such verification, the assay cannot be deemed specific for estrogenic action.

v. Because no standard protocol exists for the E-Screen assay, results generated by different investigators, and even by the same investigator, can vary widely. Several large laboratory comparisons and reviews of the E-Screen assay advocate standardization and simplification of the E-Screen. The recommendations include standardizing the MCF-7 cell line used and monitoring it for genetic drift, as well as standardizing several protocol parameters (serum clean up method, culture conditions, number of cells plated per well, estrogen-free pre-treatment period, colorimetric cell counting methods, etc.) to maximize cell proliferation responses and improve intra- and inter-laboratory reproducibility. (Sonnenschein et al. 1995, Villalobos et al. 1995, Jones et al. 1998, Korner et al. 1998, Andersen et al. 1999, Payne et al. 2000, Rasmussen & Nielsen 2002, Rajapakse et al. 2004).

We believe that the success of the validation effort and its general acceptance will depend on the transparency of the data submitted, the transparency of the evaluations made by NICEATM and ICCVAM, as well as the direct resolution of issues raised in the published literature, including discrepancies between published statements about the false positive and negative rates versus the rates listed below Table 2 in the subject document.

The Council appreciates this opportunity to provide early input on matters related to the validation of endocrine screens and tests. Please don't hesitate to call Rick Becker, Ph.D., DABT of my staff at 703-741-5210 if you have questions.

Sincerely,



Sarah H. Brozena
Senior Director
Health & Products & Science Policy

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